EASL EUROPEAN ASSOCIATION FOR THE STUDY | JOURNAL OF HEPATOLOGY

Met as a therapeutic target in HCC: Facts and hopes

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Summary

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide, and its burden is expected to increase further in the next years. In spite of the advances of classical therapies, such as surgery, transplantation, use of radiofrequency and transarterial embolization, the prognosis of this neoplasm has not considerably improved over the past few years. The advent of targeted therapies and the approval of the systemic treatment of advanced HCC with the kinase inhibitor sorafenib have provided some hope for the future. Even if the molecular mechanisms responsible for the onset and progression of HCC are still largely unknown, new therapeutic targets have recently come to the spotlight. One of these targets is the tyrosine kinase receptor for the Hepatocyte Growth Factor, encoded by the *MET* gene, known to promote tumor growth and metastasis in many human organs. In this review we will summarize the contrasting results obtained *in vitro* (in HCC cell lines) and in animal experimental models and we will also try to analyze the reasons for the opposite findings, suggesting that the HGF/MET axis can have either a promoting or a suppressive role in the development of HCC. We will also reconsider the evidence of activation of this pathway in human HCCs and discuss the results of the clinical trials performed with MET inhibitors. The final purpose is to better clarify which can be the role of MET as a therapeutic target in HCC.

Introduction

Hepatocellular carcinoma is a leading cause of cancer-related death worldwide, and its burden is expected to increase further in the next years. HCC in men is the fifth most frequently diagnosed cancer worldwide, and the second leading cause of cancer-related death, while in women it is the seventh most commonly diagnosed cancer and the sixth cause of cancer mortality [1]. The incidence of HCC varies widely, according to geographic location, and differs among racial and ethnic groups within the same country. These differences in HCC distribution are probably due to variations in exposure to hepatitis viruses and environmental pathogens.

Despite an improved treatment of viral hepatitis and an increased screening of high-risk patients in developed countries, only around 40% of HCC patients are eligible for potentially curative treatments (resection, transplantation, or local ablation) and 20% for chemoembolization. Around 40% of patients are diagnosed with advanced disease [2] and thus systemic therapy is indicated for a considerable proportion of patients. The major problems in developing effective therapies for HCC involve the intrinsic chemoresistance of HCC, the pharmacologic problems due to the presence of a diseased liver and the very advanced stage of diagnosis. Unfortunately, the efficacy of traditional chemotherapeutic agents and their ability to produce a significant survival benefit is questionable. In light of these unsatisfactory results, several studies have been performed to elucidate the molecular mechanisms underlying HCC development and progression, in order to identify targets for HCC treatment [3].

Recent progresses in the elucidation of HCC molecular pathways have brought to the clinic the multikinase inhibitor sorafenib (active against c-RAF, b-RAF, vascular endothelial growth factor receptor, c-KIT, and platelet-derived growth factor receptor beta), which has provided survival benefit in patients with advanced HCC and well-preserved liver function [4], and it is now the standard of care for patients with advanced-stage HCC [2]. However, the benefits obtained from this treatment are still disappointing and, thus, it is mandatory to find alternative effective treatments. Unlike other solid tumors, the specific sequence of genetic events that sustain hepatocarcinogenesis is unknown and, in particular, no genes to which HCC cells are "addicted" have been identified. The concept of oncogene addiction is quite recent and implies that continuous activation of specific oncogenes or inactivation of tumor suppressors is required to drive proliferation and survival of cancer cells [5]. Clinical experience has clearly shown that targeting the genes to which tumor cells are addicted can give significant therapeutic results. This is the case, for example, of Chronic Myelogenous Leukemia (CML), in which the targeted drug Imatinib inhibits the BCR-ABL tyrosine

Keywords: HGF; MET; Hepatocellular carcinoma; Targeted therapies; Clinical trials.
Received 26 June 2013; received in revised form 13 August 2013; accepted 3 September 2013
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kinase to which leukemic cells are addicted, resulting in long lasting remissions in CML patients.

Many studies have tried to identify genes or pathways to which HCC cells are addicted but, probably due to the heterogeneity of this illness, no definitive conclusion has been achieved yet. However, some genes have gained interest as possible therapeutic targets in HCC, and among them there is the receptor for Hepatocyte Growth Factor, the tyrosine kinase (TK) encoded by the *MET* gene [6]. Indeed *MET* plays a role in tumor onset and progression of different tumor types and has recently become a very interesting and studied target. Several strategies to inhibit MET activation are under development, such as tyrosine kinase inhibitors and monoclonal antibodies, and some of them are in advanced phases of clinical trials [7].

In this review we will reconsider the existing evidence for a role of MET in sustaining HCC progression and discuss if and how MET can be foreseen as a therapeutic target in this pathology.

The HGF/MET axis

The *MET* proto-oncogene encodes the tyrosine kinase receptor for Hepatocyte Growth Factor (HGF) [8,9]. Upon binding to HGF, MET becomes active and drives a complex biological program, defined as "invasive growth", resulting from the promotion of several biological activities, such as cell proliferation, cell invasion and protection from apoptosis (for a review see [10]). MET-induced invasive growth is physiologically activated during the embryonic development and in adulthood during tissue regeneration. In transformed tissues, the gain of the invasive growth program is advantageous for cancer progression and metastasis. In fact, constitutive MET activation can contribute to several aspects of tumor progression, since it forces neoplastic cells to disaggregate from the tumor mass, erode basement membranes, infiltrate stromal matrices, and eventually colonize new territories to form metastases [10].

Many works have investigated the MET-activated signaling pathways that are shared with many other receptor tyrosine kinases (RTKs), including the MAP Kinase and PI-3 Kinase-AKT pathways, STAT3, RAC1, and the NF-KB pathway (reviewed in [11]) (Fig. 1).

MET-driven signaling results from pathways directly activated by this receptor, but it can also be modulated by the cross-talk between MET and different membrane receptors, acting in complex interacting networks (Fig. 2). They involve the interaction with adhesive receptors, such as CD44 [12,13] and the $\alpha 6\beta 4$ integrin [14], with receptors for semaphorins [15], receptor tyrosine kinases, such as members of the Epidermal Growth Factor Receptor Family (EGFR and HER2) [16–18] and the Vascular Endothelial Growth Factor Receptor (VEGFR) [19] and, finally, with the pro-apoptotic receptor FAS [20]. Even if *in vitro* data suggest that these cross-talks are not essential for cell survival, they can allow a better integration of the signals present in the extracellular environment. While in physiological conditions these networks are probably redundant, it is likely that these interacting receptors cooperate in promoting tumorigenesis and/or metastasis and in inducing resistance to targeted drugs.

Data produced by many laboratories provide compelling evidence that HGF/MET signaling plays an important role in the development and progression of tumors. Indeed, (i) cell lines ectopically overexpressing *MET* or *HGF* are tumorigenic and metastatic in nude mice, whereas *MET* down-regulation decreases their tumorigenic potential [10]; (ii) *MET*- or *HGF*-transgenic mice develop metastatic tumors [21–24]; (iii) aberrant *MET* expression (usually overexpression) has been found in many kinds of solid tumors and correlates with poor prognosis [25]; (iv) the unequivocal evidence linking *MET* and human cancer comes from the presence of germline-activating mutations in patients suffering from hereditary papillary renal carcinomas [26]. Deregulated *MET* activation in cancer can be due to different molecular alterations such as overexpression, gene amplification, autocrine activation, presence of activating point mutations or downregulation of MET-targeting miRNAs [7]. While overexpression can make MET activation independent from HGF stimulation, in most cases the ligand is still required for full receptor activation [27]. This is also true for the receptor forms containing activating mutations that need HGF to fully activate their kinase activity.

HGF/MET and liver

Hepatocyte growth factor was discovered as a mitogenic protein for rat hepatocytes [28], and its cDNA was cloned in 1989 [29]. In 1991, the scatter factor and the tumor cytotoxic factor, fibroblast-derived cell molecules, were shown to be identical to HGF [30,31]. In the same year, the tyrosine kinase encoded by the *MET* gene was identified as the receptor for this growth factor [8,9].

Even if HGF was originally discovered for its mitogenic and motogenic properties, further studies revealed its ability to suppress apoptotic cell death. This cytoprotective action of HGF is responsible for liver protection from tissue damage and suppression of FAS-induced massive apoptosis of hepatocytes [32,33]. Accordingly, expression of HGF is increased in response to liver injuries, while neutralization of endogenous HGF enhances liver damage. The increased HGF production in these conditions is probably due to recruitment of bone marrow-derived liver sinusoidal endothelial cell progenitor cells [34]. The anti-apoptotic role of HGF has been clearly proven in hepatocyte conditional knockout *Met* mice, which are hypersensitive to liver injury due to treatment with agonistic anti-Fas antibodies, show delayed liver regeneration, and are more prone to liver fibrosis [35–37]. Animal studies also showed that Hgf and Met provide essential signals for survival and proliferation of hepatocytes during embryogenesis, since *Hgf* or *Met* knockout mice display considerably reduced liver size, due to decreased proliferation and increased apoptosis of hepatocytes [26,38]. These observations indicate that the HGF/MET axis is critical for liver development, protection and regeneration.

HGF/MET alterations in human HCC

On the base of the critical role of the HGF/MET axis in controlling hepatocyte proliferation and apoptosis, many studies have been performed to identify genetic and functional alterations of this signaling system in human HCC (Table 1).

As previously mentioned, in human tumors *MET* is activated by gene amplification, overexpression or activating mutations. Takeo's and Kondo's [39,40] search for *MET* amplification in HCCs revealed only a very low frequency (1 out of 20 and 1 out of 59

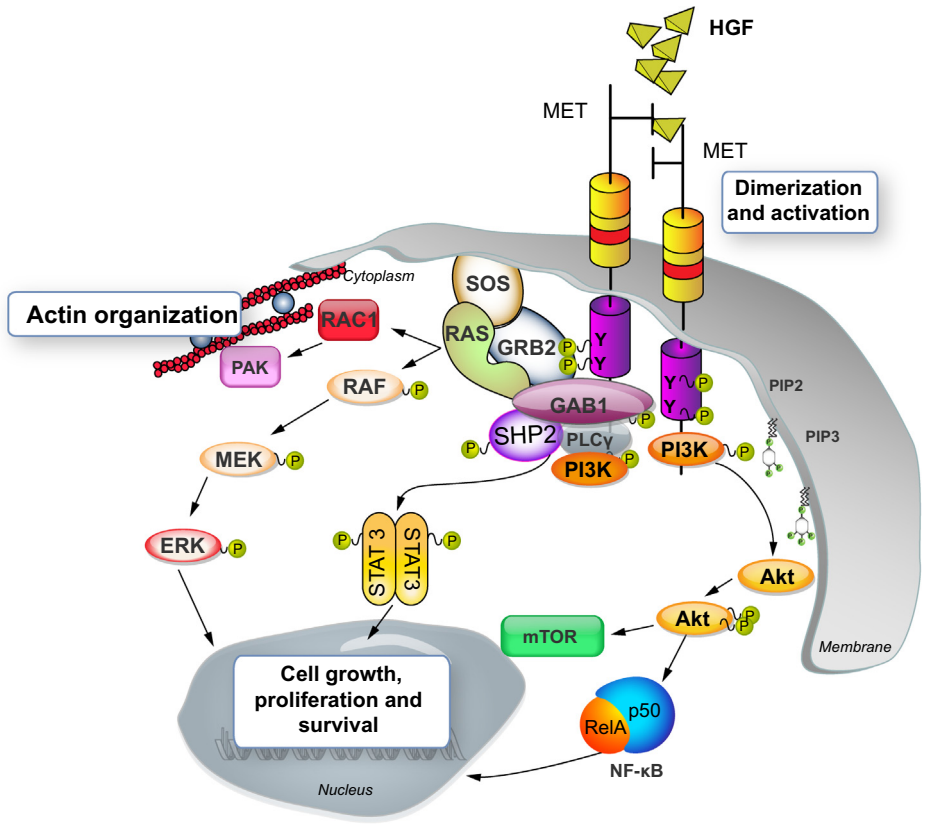

Fig. 1. MET-induced signaling pathways. Hepatocyte growth factor (HGF) promotes MET dimerization and activation. The phosphorylation of two tyrosines in the receptor tail creates binding sites for SH2-containing intracellular proteins such as Growth factor Receptor-Bound protein 2 (GRB2), Grb2-Associated Binding protein 1 (GAB1), phospholipase Cγ (PLCγ), Phosphoinositide 3-Kinase (PI3K), and Signal Transducer and Activator of Transcription 3 (STAT3). These pathways originate signals that reach the nucleus and control gene transcription and DNA replication. RAC1-dependent signals influence cytoskeletal organization.

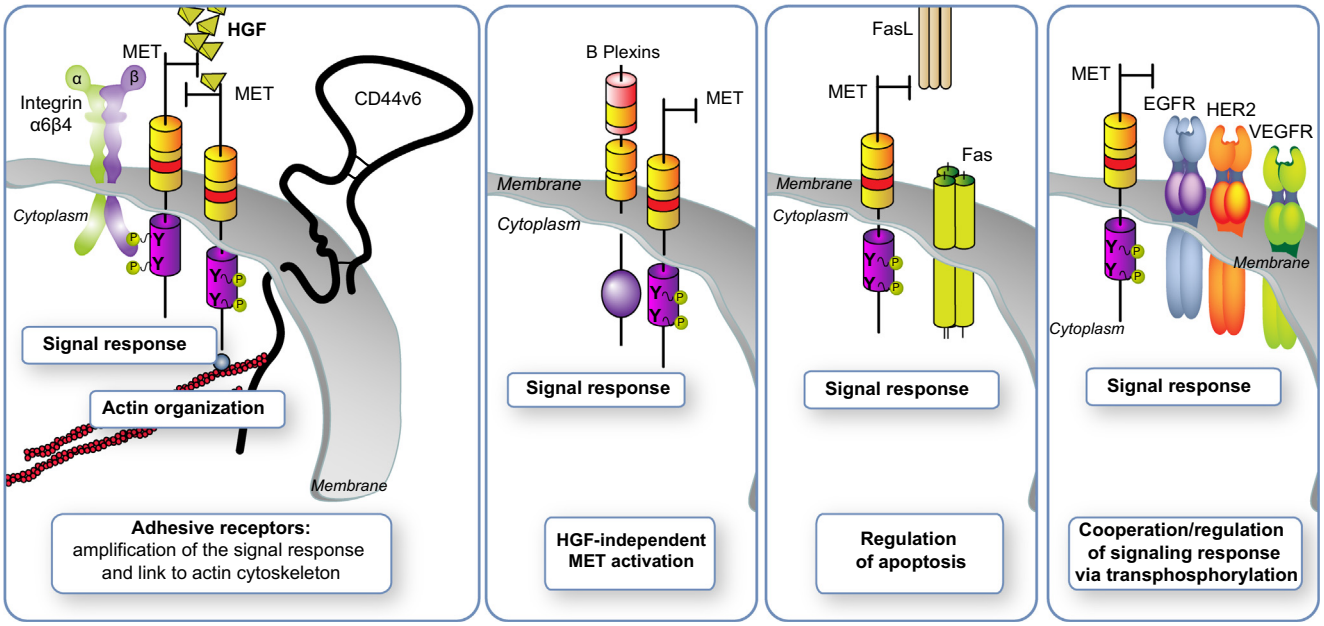

Fig. 2. MET interplay with other membrane receptors. MET-driven signaling can be modulated by cross talk with different membrane receptors. Interaction with adhesive receptors amplifies the signaling response and controls cytoskeletal reorganization; interaction with plexins (semaphorin receptors) allows HGF-independent MET activation of invasive growth; association with FAS regulates induction of apoptosis; interaction with tyrosine kinase receptors controls their reciprocal activation and (for MET-VEGFR) angiogenesis.

Table 1. Molecular alterations of HGF/MET in human HCC.

MET alteration	Findings	[Ref.]
Amplification	1/20 cases; 3.8 fold amplification	Takeo *et al.*, 2001 [39]
Amplification	1/59 cases; 22/59 chromosome 7 aneuploidy	Kondo *et al.*, 2013 [40]
Amplification	4-5% in 286 patients	Wang *et al.*, 2013 [41]
Point mutations	0/24 patients	Guichard *et al.*, 2012 [42]
Overexpression	Northern blot analysis; overexpression in 8/18 cases with 2-10 fold increase compared with the surrounding liver	Boix *et al.*, 1994 [45].
Overexpression	Northern blot analysis: 6/19 cases; 16/23 with IHC. Correlation with poor to moderate HCC differentiation	Suzuki *et al.*, 1994 [46]
Overexpression	Competitive RT-PCR. Overexpression in some of the 11 patients. HGF undetectable	Noguchi *et al.*, 1996 [47]
Overexpression	Northern blot analysis. Met overexpression in some cases and underexpression in others. HGF downregulation	Selden *et al.*, [89]
Overexpression	Western blot analysis. 52% of 62 patients with Met overexpression, correlating with increased incidence of intrahepatic metastases and shorter 5-yr OS	Ueki *et al.*,1997 [44]
Overexpression	IHC in 86 patients. MET overexpression in 20% and downregulation in 32%. HGF overexpression in 33% and downregulation in 20%	Kiss *et al.*, 1997 [48]
Overexpression	IHC and qRT-PCR in 24 HCC. MET overexpression in most of the cases. Underexpression of HGF	Tavian *et al.*, 2000 [49]
Overexpression	qRT-PCR in 15 patients. Overexpression of MET in poorly differentiated tumors	Daveau *et al.*, 2003 [50]

IHC, immunohistochemistry; OS, overall survival; qRT PCR, quantitative Reverse Transcription Polymerase Chain Reaction.

HCCs, respectively); however, aneuploidy of chromosome 7 (where both *MET* and *HGF* are located) was present in 22/59 patients. A very recent work by Wang *et al.* [41] examined the genomic landscape of copy number aberrations in 286 hepatocarcinoma patients and identified recurrently amplified regions with a high level of copy number changes. *MET* was identified as one of the ten genes in the amplification peak located at 7q31.2, present in 4–5% of the cases. No amplification of HGF, located on the same chromosome but in a different region, was found. Concerning activating point mutations, Guichard and colleagues [42], who performed the whole exome sequencing on 24 tumors, did not identify any activating mutation in *MET*.

Over the past few years, expression of MET and HGF (produced by stromal components, cancer-associated fibroblasts and endothelium in the tumor mass [43,44]) has been evaluated in many studies. In small groups of patients (18 and 19, respectively) [45,46] Northern blot analysis showed an increase of MET mRNA in 30–40% of HCCs compared to peritumoral tissue. While Boix *et al.* did not find any correlation with clinical parameters, Suzuki *et al.* observed an association between MET overexpression and poor-to-moderate differentiation of cancer cells and a non-significant increase in the proliferative activity of tumor cells. By competitive PCR, Noguchi and collaborators [47] found that MET expression was increased in some cases of HCC, while HGF was expressed at levels lower than those of the peritumoral tissue. By western blot analysis, Ueki *et al.* [44] found MET overexpression in 48% of 62 HCC patients, correlating with an increased incidence of intrahepatic metastases. Patients with high MET HCC had a significantly shorter 5-year survival than patients with low MET HCC (33.5% *vs.* 80.3%, respectively; $p < 0.05$). However, they did not find any correlation between HGF concentration in the tumor tissue, clinic pathological factors and patient survival. Kiss *et al.* [48] analyzed 86 HCCs by immunohistochemistry and found MET overexpression in 20% and downregulation in 32% of cases, while HGF was increased in 33% and decreased in 21% of tumors. Tavian *et al.* [49] performed RT-PCR on 24 HCCs and found overexpression of MET and underexpression of HGF compared to the corresponding peri-tumoral tissues. HGF and MET levels did not correlate with clinical features, but increased MET was inversely associated with patient survival. Finally, Daveau *et al.* [50] performed quantitative RT-PCR on 15 HCCs and peritumoral tissues and found low levels of HGF in highly differentiated tumors, whereas overexpression of MET was observed in poorly differentiated tumors and in patients with early tumor recurrence.

As it can be seen by the overall analysis of these works, while most of the studies agree on the decrease of HGF in HCC, it is difficult to draw a definitive picture on the status and role of MET in liver cancer. In fact, there is not only disagreement on the percentage of tumors showing MET overexpression, but opposite results are often present in the literature. Which are the explanations for these discrepancies? One possible reason is the use of different techniques (Northern blot, Western blot, competitive PCR, RT-PCR), which have different sensitivity and different modality of quantification. Moreover, even when the same technique is used, there is no agreement on the adopted scoring system. Another possible reason is that many of these studies have been performed on small groups of patients and, due to the different etiologies of HCC, they could have included unbalanced types of tumors. Most importantly, none of these studies investigated the activation status of MET, which is critical to identify tumors that can benefit from anti-MET drugs. As *MET* amplification or mutation seem to be very rare, the only criterium to select patients for possible anti-MET therapies is overexpression, but – at the moment – there is no standardized test to identify a level of expression that could render tumor cells "addicted" to MET. This is even more important if we think that most of the studies found decreased HGF levels in HCC, suggesting that MET activation has to be largely ligand-independent, such as that due to very high levels of overexpression. Alternatively, as shown by two studies [51,52], MET could be activated by the binding with des-gamma-carboxy prothrombin (DCP), a well-recognized tumor marker recently exploited due to its high sensitivity and specificity in the screening and diagnosis of hepatocellular

carcinoma. DCP is elevated in the serum of 44–81% of HCC patients [53] and was shown to be able to bind MET, causing its autophosphorylation and the proliferation of HCC cells [51]. Even if further studies are required to draw a final conclusion, it is thus possible that in many HCCs MET activation is not due to HGF but to the autocrine/paracrine production of DCP.

A different approach to investigate the role of MET activation in human tumors was taken by Kaposi-Novak and colleagues [54]. Using global gene expression profiling of WT and *Met*-deficient primary mouse hepatocytes, the authors defined a Met-dependent gene expression signature. To assess the importance of this signature, they applied a comparative functional genomic approach to 242 human HCCs and liver metastases. The analysis revealed that a subset of human HCCs and all liver metastases shared the Met-driven expression signature, which correlated with increased vascular invasion rate, microvessel density and decreased mean survival time of HCC patients. Thus, they concluded that Met-driven expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype.

In vitro preclinical data of HGF/MET inhibition/overexpression

Many *in vitro* studies have evaluated the effect of the activation of the HGF/MET axis in HCC cell lines. Interestingly, the first studies aimed at demonstrating a pro-proliferative role of HGF in liver tumor cells, as observed in normal hepatocytes, showed a marked inhibition of cell growth [55,56]. Growth inhibition was also observed in HCC cells stably transduced with HGF. However, HGF stimulation resulted in increased invasive properties of tumor cells [56,57]. The biochemical mechanisms responsible for the concomitant anti-proliferative and pro-invasive role of HGF in HCC cells are not fully elucidated. Shirako *et al.* [58] have shown in HepG2 cells that the growth inhibitory activity of HGF requires a strong activation of ERK and up-regulation of p16 and p21 expression, which contributes to the suppression of Cdk2 activity.

On the other hand, different studies have shown that MET down-regulation or inhibition interferes with both cell growth and cell invasiveness [59–61]. The reasons for the different behavior of HGF in normal *vs.* transformed hepatocytes and for the similar effects caused by HGF stimulation and MET inhibition in HCC remain elusive. One possibility could be the availability of other ligands able to activate MET in the liver. Suzuki *et al.* [51] identified such a molecule in des-gamma-carboxy prothrombin. DCP contains two kringle domains similar to those of HGF, required for HGF binding to MET. They showed that DCP binds MET and that this interaction promotes proliferation of HCC cell lines and induces JAK1/STAT3 activation, without affecting the Raf/MAPK and the PI3K/AKT pathways. This study thus suggests that the HCC cells can autocrinally activate MET signaling by producing DCP, whose expression is a bad prognostic indicator. If this holds true, it remains to be explained why the biological effects of DCP/MET interaction are opposite to those elicited by HGF/MET binding in the same cells.

As mentioned above, several studies have shown that MET can interact with different tyrosine kinase receptors; in particular, a functional cross-talk has been demonstrated between MET and the different members of the EGFR family [16–18]. Indeed, Met associates with EGFR in tumor cells, and this association results in MET phosphorylation in the absence of HGF [16]. It can thus be hypothesized that MET phosphorylation in HCC cells could also be due to constitutive activation of EGFR family members, whose alterations in liver cancers have been reported [3]. The demonstration of a functional and biologically meaningful cross talk between MET and EGFR in HCC cells would pave the way to the use of combination therapies targeting both receptors, which are already in clinical trials in other tumor types.

In vivo preclinical data of MET/HGF manipulation

The critical role of the HGF/MET axis in development was well illustrated by the effects exerted by knock-out of either *Met* or *Hgf* in mice. *Hgf*- and *Met*-null mutant embryos fail to complete development and die in utero [62,63]. The mutation affects the embryonic liver, which is reduced in size and shows extensive loss of parenchymal cells.

Several works examined the effects of genetic inactivation or overexpression of either *Met* or *Hgf* on hepatocarcinogenesis. The published results, however, are discordant and the reasons for these discrepancies are not yet clear (Table 2).

Two works examined the effect of liver-specific *Met* knock out in hepatocarcinogenesis [64,65]. The loss of Met signaling in hepatocytes increased rather than suppressed tumor initiation by DEN (N-nitrosodiethylamine), as the animals developed significantly more and bigger tumors and with a shorter latency, compared to controls. The authors suggested that this was probably due to the loss of the physiological role of Met in maintaining normal redox homeostasis in the liver and, thus, the lack of this activity resulted in a tumor suppressive role of Met in this context. On the contrary, a protumorigenic role was observed when *Met* was hydrodynamically transfected in the liver or in *Met* transgenic mice [66].

In the same manner, both stimulatory and inhibitory effects of exogenous administration of HGF on carcinogen-treated rats have been reported [56,67,68]. In fact, while injection of HGF to rats initiated with DEN and promoted with N-ethyl-N-hydroxyethylnitrosamine significantly increased the development of preneoplastic foci [68], Liu *et al.* observed a strong inhibitory activity of HGF in rat liver tumors induced by DEN [67]. Finally, Ogasawara *et al.* found that HGF promotes proliferation of preneoplastic hepatocytes but does not affect the growth of liver carcinoma cells in 3′-Me-DAB-treated rats [56].

Reports obtained in *HGF* transgenic mice are conflicting as well. A pro-tumorigenic role for HGF was clearly suggested by Sakata *et al.* [21], who generated *Hgf* transgenic mice (under the control of the mouse metallothionein gene promoter); they found that in adult transgenic animals, liver weight (as a percentage of total body weight) was at least twice the weight of wild-type mice and the DNA labeling index of hepatocytes was significantly increased. Moreover, this proliferative stimulus triggered the formation of hepatocellular adenomas and/or carcinomas in most transgenic mice older than 1.5 years. In contrast, overexpression of a human *HGF* cDNA under the control of the albumin promoter did not induce HCC [69]. These different results can be due to at least two reasons: (i) the level of Hgf expression achieved in the serum through the use of the metallothionein promoter was 2–5-fold higher compared to that obtained with the albumin promoter; (ii) Sakata used a mouse *Hgf* cDNA instead of the human *HGF* cDNA utilized by Shiota; it is known that the cross interaction between human HGF and mouse Met is not adequate to promote the optimal activity of the receptor.

A pro-tumorigenic role of HGF was found in transgenic mice also by Horiguchi *et al.* [70], who observed accelerated DEN-induced hepatocarcinogenesis, often accompanied by abnormal

Table 2. *In vivo* manipulation of HGF/MET.

In vivo manipulation	Findings	[Ref.]
HGF KO	Impaired development of embryonic liver	Uehara *et al.*,, 1995 [63]
HGF injection	DEN treated mice showed a significant increase of liver preneoplastic foci	Yaono *et al.*, 1995 [68]
HGF infusion	Strong inhibition of DEN induced liver tumors	Liu *et al.*, 1995 [67]
HGF injection	Increased growth of preneoplastic hepatocytes but no effect on liver carcinoma cell growth in 3'-Me-DAB-treated rats	Ogasawara *et al.*, 1998 [56]
HGF transgenic mice (albumin promoter)	No HCC	Shiota *et al.*, 1994 [69]
HGF transgenic mice (metallothionine promoter)	HCC and adenomas in mice older than 1.5 yr	Sakata *et al.*, 1996 [21]
HGF transgenic mice (metallothionine promoter)	Accelerated DEN-induced hepatocarcinogenesis	Horiguchi *et al.*, 2002 [70]
HGF/*MYC* transgenic mice	HGF transgene delayed the appearance of preneoplastic lesions and prevented malignant conversion compared to MYC alone transgenic mice	Santoni-Rugiu *et al.*, 1996 [71]
HGF/*TGFα* transgenic mice	HGF transgene decreased hepatocarcinogenesis compared to TGFα alone transgenic mice	Shiota *et al.*, 1994 [72]
MET KO	Impaired development of embryonic liver. MET null embryonic stem cells do not contribute to adult liver	Schmidt *et al.*, 1995 [62]
Liver-specific *MET* KO	Accelerated DEN-induced hepatocarcinogenesis	Takami *et al.*, 2007 [64]
Liver-specific *MET* KO	Accelerated DEN-induced hepatocarcinogenesis	Marx-Stoelting *et al.*, 2009 [65]
Liver-specific transgenic mice	HCC development by 3 mo of age	Tward *et al.*, 2005 [66]
MET hydrodynamic transfection in the liver	HCC development in 74% of mice transfected with MET and active β-catenin	Tward *et al.*, 2005 [66]

DEN, Diethylnitrosamine; TGFα, Transforming Growth Factor α; 3′-Me-DAB, 3′-Methyl-4-dimethylaminoazobenzene.

blood vessel formation. Thirty-three percent of males and 23% of female transgenic mice developed hepatocellular carcinoma, while none of the wild-type mice developed HCC. Since the authors also detected enhanced *Met* kinase activity in most of these tumors, they concluded that HGF promotes hepatocarcinogenesis through the autocrine activation of the HGF/MET signaling pathway, in association with stimulation of angiogenesis.

On the contrary, an inhibitory role of HGF in liver carcinogenesis was demonstrated in double transgenic animals, where the *Hgf* transgene inhibited hepatocarcinogenesis in mice overexpressing c-*myc* [71] and transforming growth factor alpha (TGFα) [72].

Therefore, in light of these results, the issue of the pro-tumorigenic role of HGF and MET in the liver remains to be resolved.

MET as an anti-angiogenic target

Several experimental works showed, *in vitro* and *in vivo*, that the HGF/MET axis promotes angiogenesis. HGF is an angiogenic factor able to activate MET in endothelial cells, in part through a direct effect [73], in part by inducing Vascular Endothelial Growth Factor (VEGF) production and decreasing thrombospondin-1 expression [74]. Moreover, very recently, it has been shown that VEGFR and MET physically interact and that VEGF enhances recruitment of the protein tyrosine phosphatase 1B to a MET/VEGFR2 heterocomplex, thereby suppressing HGF-dependent MET phosphorylation and activation [19]. Consequently, VEGF blockade restores and increases MET activity.

Following the initial observations that anti-angiogenic molecules could function as potent tumor suppressors in mice, several therapeutic strategies have attempted to interfere with tumor growth by suppressing neo-angiogenesis. However, it has become clear that anti-angiogenic therapies induce tumor hypoxia that, in turn, allows for selection of more aggressive tumor cells. MET transcription is induced by the hypoxia-inducible factor HIF-1α in tumor cells [75,76], and MET expression was found to be upregulated following anti-angiogenic therapies [77]. Thus, antiangiogenic therapies can interfere with MET activation in a dual way: (i) by promoting hypoxia-induced MET-expression and (ii) by promoting MET activity as a consequence of VEGF blockade.

Some studies have evaluated the effect of the simultaneous inhibition of MET and VEGFR pathways in HCC. Treatment of SK-HEP1 cells with foretinib (an oral multikinase inhibitor targeting MET, RON, AXL, TIE-2, and VEGFR2 receptors) resulted in growth inhibition, G2/M cell cycle arrest, reduced colony formation and blockade of HGF-induced cell migration [78]. In animal models of HCC, foretinib potently inhibited tumor growth and inhibition of angiogenesis correlated with inactivation of VEGFR2/MET signaling pathways. Another study was performed on 20 HCC patients, treated with tivantinib (a small kinase MET inhibitor) plus sorafenib [79]. Overall response rate and disease control rate were 10% and 70%, respectively. Best response was 1 complete response (CR), 1 partial response (PR), and 12 stable diseases (SD). Among 8 patients previously treated with VEGFR inhibitors (6 sorafenib; 1 sunitinib; 1 sorafenib plus sunitinib), best response was 1 CR, 1 PR, and 3 SD. This study thus suggests that the combination of tivantinib plus sorafenib may have therapeutic potential in HCC patients, including those pretreated with VEGFR inhibitors.

The HGF/MET axis as a clinical target in HCC

Although there are no approved anti-*Met* agents, a number of MET/HGF inhibitors have been or are currently under study in trials in cancer patients [7]. Different molecules like monoclonal

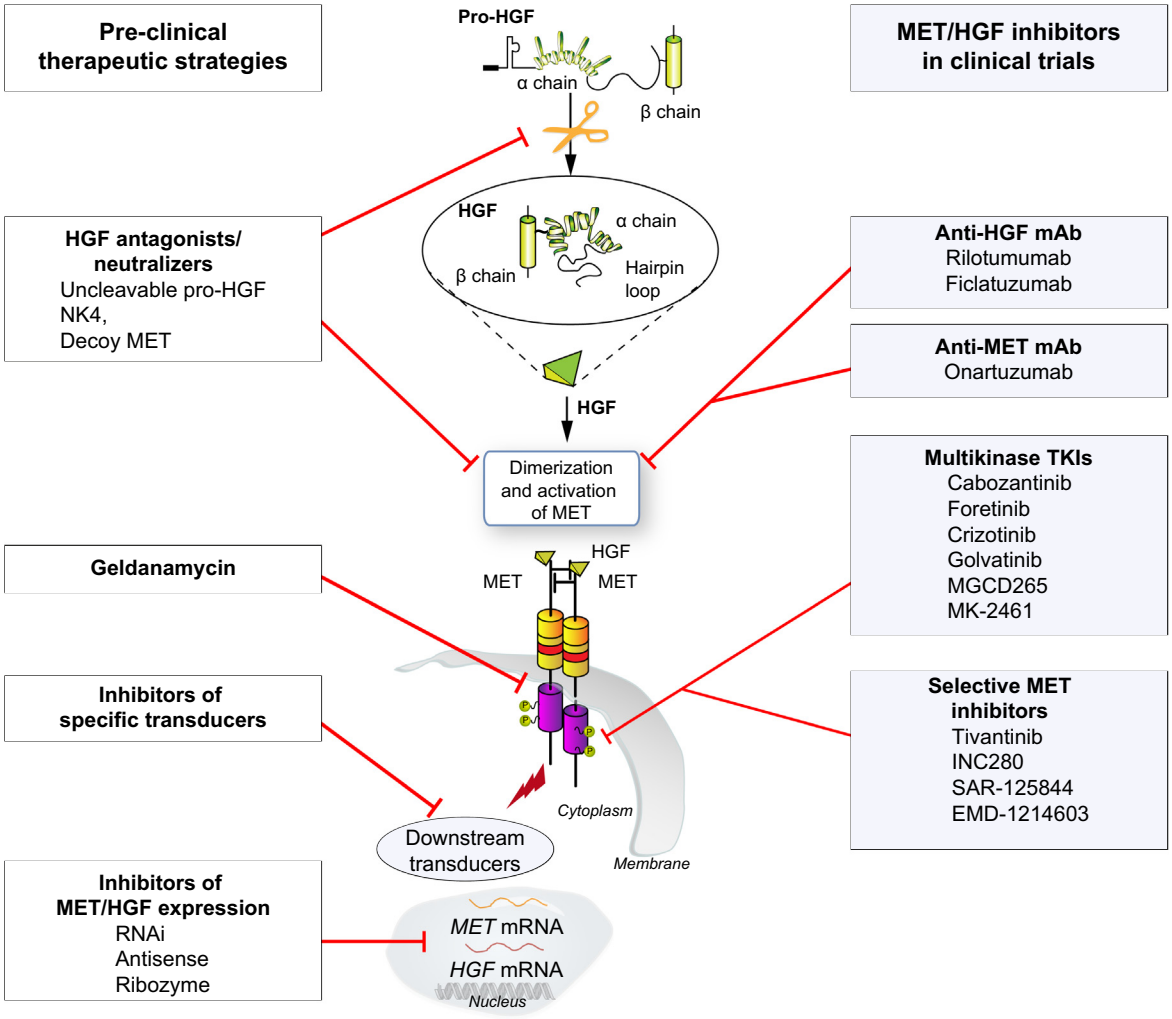

Fig. 3. Strategies to target MET signaling. The central panel shows the physiological activation pathway of MET: pro-HGF is converted into mature HGF that binds to the receptor and induces its dimerization and activation. Schematic MET structure: sema domain: yellow rectangle; MET-related sequence: orange rectangle; tyrosine kinase domain (purple); (P): tyrosines of the intracellular docking site. The left side of the figure shows the therapeutic strategies utilized in preclinical studies. The upper square illustrates molecules that act as HGF antagonists (NK4, uncleavable pro-HGF and decoy MET). Geldanamycin induces MET ubiquitination and proteasomal degradation. Specific inhibitors (SH2 competitors and inhibitors of specific downstream molecules, such as Src, PtdIns3K, MAPK or STAT3) block critical transducers. The lower square shows the mechanisms aimed at silencing MET or HGF expression (antisense oligonucleotides, ribozymes and RNAi). The right side illustrates MET/HGF inhibitors in clinical trials. Anti-HGF and anti-MET antibodies prevent ligand-induced activation. Many non-selective and selective small molecules inhibit MET kinase activity. Red lines indicate the site of action of each drug.

antibodies (mAbs) against HGF or MET and specific/non-specific MET inhibitors are now available, but most of them are still at early stages of clinical development (Fig. 3); the data reported so far have shown some clinical benefits in patients with various tumor types.

Concerning HCC, three TKIs have been used in Phase II clinical trials: tivantinib, foretinib and cabozantinib (Table 3).

Tivantinib, a staurosporine derivative showing promising activity in a variety of phase I/II clinical trials, was initially reported as a selective, non-ATP competitive MET inhibitor [80]. This is the drug at the most advanced stage of clinical testing in HCC; in fact, a Phase III trial (METIV) is ongoing, on the basis of the results obtained in a phase II, double-blind, placebo-controlled clinical trial [81]. This trial assessed the efficacy of tivantinib in patients with advanced-stage HCC and Child-Pugh A cirrhosis, who had progressive disease during first-line therapy (72 patients were treated with tivantinib and 36 were assigned to the placebo arm). The primary end-point was time to progression; the trial also included the immunohistochemistry assessment of MET expression in the tumors. Time to progression was longer for patients in the tivantinib arm than for those in the placebo arm (1.6 months *vs.* 1.4 months; $p = 0.04$). The results were more positive in patients with high expression of MET, showing a median time to progression of 2.7 months *vs.* 1.4 months of the placebo arm. Although these results seem encouraging, two recent works [82,83] question the mechanism of action of the drug, as they show that tivantinib acts on microtubule dynamics independently of MET and thus it behaves more as a cytotoxic rather than a targeted drug. In fact, the authors of these two papers provided evidence that (i) tivantinib inhibits the growth of both MET-dependent and independent cancer cells, (ii) it is active on cells not expressing MET, (iii) it does not inhibit

Table 3. Clinical trials targeting MET in HCC.

Drug	Targets	Phase and dosage	Eligibility	Results	Adverse effects
Cabozantinib [88]	VEGFR2, MET, RET, KIT, FLT4, AXL	Phase II 100 mg/die	41 pts Child-Pugh Score A	2 PR and 32 SD. mPFS: 4.4 mo; mOS: 15.1 mo. Activity irrespective of sorafenib pretreatment status	Frequent grade 3-4 adverse effects: diarrhea, palmar-plantar erythrodysestesia syndrome, thrombocytopenia
Foretinib [87]	MET, VEGFR2, TIE2, FLT4; RON, FLT3, KIT, FLT1, PDGRFβ	Phase I/II 30 mg/die	39 pts Child-Pugh Score A; no prior sorafenib or TKIs	RR: 24% SD: 58% mTTP: 4.2 mo mOS: 15.7 mo	Grade 3-4 adverse effects in ≥5% pts : hypertension, ascites, increased ALT, abdominal pain, hypoalbuminemia, hyponatriemia
Tivantinib [81]	MET	Phase II 360 mg twice-daily or 240 mg twice daily or placebo	107 pts Child-Pugh Score A	Tivantinib *vs.* placebo: TTP: 1.6 *vs.* 1.4 mo PFS 1.7 *vs.* 1.5 mo; OS 6.6 (7.5 in patients treated with 240 mg) *vs.* 6.2 mo In patients with MET-high tumors, TTP 2.7 *vs.* 1.4 mo PFS: 2.2 *vs.* 1.4 mo. OS: 7.2 *vs.* 3.8 mo	Grade 3-4 adverse effects in ≥5% pts: neutropenia, anemia, fatigue, thrombocytopenia, leucopenia, bradycardia, diarrhea, vomiting, nausea, febrile neutropenia, pancytopenia, sepsis neutropenic sepsis, 4 deaths from severe neutropenia
Tivantinib + sorafenib [79]		Phase I 240 or 360 mg Tivantinib twice-daily + 400 mg sorafenib twice daily	20 pts Child-Pugh Score A, B	1 CR, 1 PR and 12 SD RR: 10% DCR: 70% mPFS: 3.5 mo	Adverse effects ≥25%: rash, palmar-plantar erythrodysestesia syndrome, fatigue, diarrhea, nausea, anorexia
INC280		Phase II	Patients with HCC and MET pathway dysregulation	ongoing	
Tivantinib		Phase III 240 mg twice daily or placebo	Patients with HCC and high MET expression	ongoing	

SD, Stable disease; mPFS, medium Progression Free Survival; mOS, medium Overall Survival; TKIs, Tyrosine Kinase Inhibitors; mTTP, medium Time To Progression; CR, Complete Response; PR, Partial Response; RR, Response Rate; DCR, Disease Control Rate.

MET activation in addicted cells. Altogether these results put a strong caveat on the real ability of tivantinib to specifically target MET. This does not imply that the drug is ineffective, but rather that it may act through a mechanism different from the one hypothesized. In the past, other "targeted drugs" turned out to be "cytotoxic drugs". A notable example is iniparib, which gave positive results in triple-negative breast cancer in a phase II trial [84], but later on turned out not to be a PARP (poly-(ADP-ribose) polymerase) inhibitor but rather a cytotoxic agent [85,86]. Overall, the results of the study by Santoro *et al.* should be taken with caution, while waiting for the results of the Phase III METIV trail, enrolling only HCC patients with high MET expression to be treated with Tivantinib.

Foretinib is an oral multikinase inhibitor targeting MET, RON, AXL, TIE-2, and VEGFR2. Yau *et al.* reported the results of a phase I/II trial (MET111645), evaluating oral foretinib as first line therapy in advanced Asian HCC patients [87]. Thirty-nine patients were enrolled and thirty-eight were evaluable for efficacy. The primary endpoint was safety and tolerability at the maximum tolerated dose and the secondary endpoint included antitumor activity (objective response rate, disease stabilization rate, time to progression and overall survival). The overall response rate was 24%, disease stabilization rate 79% and the median time to progression was 4.2 months. The most common adverse effects were hypertension (36%), decreased appetite (23%), and pyrexia (21%). The most common serious adverse effects were hepatic encephalopathy (10%) and ascites (8%).

Cabozantinib is an oral inhibitor of MET, VEGFR, and RET. A phase II randomized discontinuation trial was performed by Verslype *et al.* [88] on 41 patients with advanced HCC and Child-Plugh cirrhosis. After 12 weeks, only patients with a partial response were maintained on open-label cabozantinib, while patients with stable disease were randomized to either cabozantinib or placebo. The primary endpoint during the randomization phase was overall response rate. Median PFS from the first day of study was 4.2 months. Two out of 36 patients evaluable for tumor assessment at 12 weeks achieved a confirmed PR. One more patient randomized at week 12 achieved a PR at 18 weeks. Twenty eight out of 36 patients (78%) with $\geqslant 1$ post-baseline scan had tumor regression. The overall disease control rate at week 12 was 68%. A reduction of alpha fetoprotein (AFP) >50% in patients with elevated AFP at baseline was observed in 10/26 patients (38%). Interestingly, previous treatment with sorafenib did not influence PFS.

At the moment, another anti-MET drug, INC280 by Novartis, is in Phase II clinical trial, in HCC patients with MET pathway dysregulation.

Conclusions

HGF was originally discovered for its ability to promote growth of hepatocytes and thus, from the beginning, its involvement in the development and progression of liver tumors was considered quite likely. However, the studies performed both *in vitro* – in HCC cell lines – and in animal models gave contradictory results, suggesting either an oncogenic or a suppressive role in liver cancer for the HGF/MET axis. The reasons for the observed differences are not clear. It can be hypothesized that MET overactivation can indeed promote signaling of HCC cells, but it is not clear how this receptor can be activated in tumor cells, since almost all the data show that HGF-mediated MET activation leads to inhibition of tumor growth. Several mechanisms of HGF-independent MET activation have been described in different conditions, but they have not been investigated in the context of liver tumors, with the exception of the possible role of des-gamma-carboxiprotrombin as an alternative MET ligand. Concerning the studies that have shown that the loss of HGF/MET signaling can accelerate chemically-induced carcinogenesis, it is possible – as suggested by Takami – that MET-mediated signaling is necessary to maintain normal redox homeostasis in the liver, leading to the identification of an oncosuppressive role for this gene [64].

The clinical data obtained with MET inhibitors in HCC are not very encouraging either. A further complication to the scenario is that the best results have been obtained with a drug whose specific activity against MET has recently been questioned [82,83].

The main problems of the performed clinical studies are the relatively small number of patients recruited and the lack of selection for patients displaying activation of the MET signaling pathway. The experience obtained from the studies performed with different targeted drugs in other tumors has taught that they are effective almost exclusively in cells addicted to the gene targeted by the drug. If we look, for example, at non-small cell lung cancer, EGFR inhibitors are effective when EGFR mutations are present, while crizotinib treatment is very active only in patients with ALK translocation. Since these latter patients are 3% of total NSCLCs, a study with crizotinib on the whole population of NSCLC patients would securely lead to negative results, with the risk of leaving apart a drug that could be active on a selected population. A similar situation can occur in the case of HCC, where patient selection based on the activation of the MET pathway, a crucial step in targeted therapy, has never been done. Activation due to gene amplification or activating mutations is probably infrequent, since studies evaluating those alterations in the whole genome have revealed rare anomalies in the MET gene. The situation is more complex when overexpression is considered. Some studies have indeed revealed different degrees of overexpression, but the results are very heterogeneous and cannot be compared. Moreover, no proof has been given that the observed overexpression can lead to constitutive MET pathway activation. These evaluations, however, open new questions because, with the currently available IHC protocols, there are no validated techniques to detect MET phosphorylation and HGF expression in tumor samples.

Even if the role of HGF/MET in HCC remains elusive, there are still reasons suggesting that MET can be considered an interesting target in HCC. However, to reach a definitive conclusion, more focused and wider trials, carefully investigating the status of MET and of its signaling pathway in patients' tumors, are mandatory. Careful identification of patients likely responsive to MET targeted drugs will also allow clinical studies evaluating the efficacy of combination with chemotherapeutic agents or other targeted therapies (such as sorafenib or EGFR-targeted drugs). Clinical experience has, in fact, shown that these combinations are often more effective than the molecular drugs given alone. If the results show that indeed MET inhibition is therapeutically effective in HCC, a novel field will open: the search of targeted drugs that can be safely used in a population, such as liver cancer patients, in which liver functionality is often poor.

Key Points

- The risk to develop HCC is constantly increasing and the molecular mechanisms underlying its development are still poorly understood. In spite of the advances of classical therapies and of novel targeted therapies, the prognosis of this neoplasm has not considerably improved over the past few years
- MET has been proposed as one of the targets of these therapies. A deeper understanding of the role of MET as a therapeutic target in HCC is, however, required, in view of the contradictory data about the role of MET and its ligand HGF in HCC development
- *In vitro* data show that HGF promotes proliferation of normal hepatocytes but inhibits growth of HCC cells. Several mechanisms of HGF-independent MET activation have been described, but they have not been investigated in depth in the context of liver tumors
- On the base of preclinical data, the issue of the protumorigenic role of HGF and MET in the liver remains to be resolved
- More focused and wider trials, investigating the status of MET and of its signaling pathway in patients' tumors, are needed to gauge the efficacy of anti-Met selective therapies

Financial support

This work was supported by Associazione Italiana Ricerca sul Cancro (AIRC, Grants IG-11821 to AC and IG-11819 to SG).

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgments

We thank Dr. F. Natale for editing the manuscript.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.

[2] Llovet JM, Di Bisceglie AM, Bruix J, Kramer BS, Lencioni R, Zhu AX, et al. Design and endpoints of clinical trials in hepatocellular carcinoma. J Natl Cancer Inst 2008;100:698–711.
[3] Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008;48:1312–1327.
[4] Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.
[5] Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell 2009;136:823–837.
[6] Giordano S, Ponzetto C, Di Renzo MF, Cooper CS, Comoglio PM. Tyrosine kinase receptor indistinguishable from the c-met protein. Nature 1989;339:155–156.
[7] Corso S, Giordano S. Cell autonomous and non-cell autonomous mechanisms of HGF/MET-driven resistance to targeted therapies: from basic research to a clinical perspective. Cancer Discov 2013;3:978–992.
[8] Bottaro DP, Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, et al. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. Science 1991;251:802–804.
[9] Naldini L, Vigna E, Narsimhan RP, Gaudino G, Zarnegar R, Michalopoulos GK, et al. Hepatocyte growth factor (HGF) stimulates the tyrosine kinase activity of the receptor encoded by the proto-oncogene c-MET. Oncogene 1991;6:501–504.
[10] Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF. Met, metastasis, motility and more. Nat Rev Mol Cell Biol 2003;4:915–925.
[11] Migliore C, Giordano S. Molecular cancer therapy: can our expectation be MET? Eur J Cancer 2008;44:641–651.
[12] van der Voort R, Taher TE, Wielenga VJ, Spaargaren M, Prevo R, Smit L, et al. Heparan sulfate-modified CD44 promotes hepatocyte growth factor/scatter factor-induced signal transduction through the receptor tyrosine kinase c-Met. J Biol Chem 1999;274:6499–6506.
[13] Orian-Rousseau V, Chen L, Sleeman JP, Herrlich P, Ponta H. CD44 is required for two consecutive steps in HGF/c-Met signaling. Genes Dev 2002;16:3074–3086.
[14] Trusolino L, Bertotti A, Comoglio PM. A signaling adapter function for alpha6beta4 integrin in the control of HGF-dependent invasive growth. Cell 2001;107:643–654.
[15] Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, et al. The semaphorin 4D receptor controls invasive growth by coupling with Met. Nat Cell Biol 2002;4:720–724.
[16] Jo M, Stolz DB, Esplen JE, Dorko K, Michalopoulos GK, Strom SC. Cross-talk between epidermal growth factor receptor and c-Met signal pathways in transformed cells. J Biol Chem 2000;275:8806–8811.
[17] Bergstrom JD, Westermark B, Heldin NE. Epidermal growth factor receptor signaling activates met in human anaplastic thyroid carcinoma cells. Exp Cell Res 2000;259:293–299.
[18] Fischer OM, Giordano S, Comoglio PM, Ullrich A. Reactive oxygen species mediate Met receptor transactivation by G protein-coupled receptors and the epidermal growth factor receptor in human carcinoma cells. J Biol Chem 2004;279:28970–28978.
[19] Lu KV, Chang JP, Parachoniak CA, Pandika MM, Aghi MK, Meyronet D, et al. VEGF inhibits tumor cell invasion and mesenchymal transition through a MET/VEGFR2 complex. Cancer Cell 2012;22:21–35.
[20] Wang X, DeFrances MC, Dai Y, Pediaditakis P, Johnson C, Bell A, et al. A mechanism of cell survival: sequestration of Fas by the HGF receptor Met. Mol Cell 2002;9:411–421.
[21] Sakata H, Takayama H, Sharp R, Rubin JS, Merlino G, Larochelle WJ. Hepatocyte growth factor/scatter factor overexpression induces growth, abnormal development, and tumor formation in transgenic mouse livers. Cell Growth Differ 1996;7:1513–1523.
[22] Wang R, Ferrell LD, Faouzi S, Maher JJ, Bishop JM. Activation of the Met receptor by cell attachment induces and sustains hepatocellular carcinomas in transgenic mice. J Cell Biol 2001;153:1023–1034.
[23] Takayama H, Larochelle WJ, Sharp R, Otsuka T, Kriebel P, Anver M, et al. Diverse tumorigenesis associated with aberrant development in mice overexpressing hepatocyte growth factor/scatter factor. Proc Natl Acad Sci U S A 1997;94:701–706.
[24] Liang TJ, Reid AE, Xavier R, Cardiff RD, Wang TC. Transgenic expression of tpr-met oncogene leads to development of mammary hyperplasia and tumors. J Clin Invest 1996;97:2872–2877.
[25] Comoglio PM, Giordano S, Trusolino L. Drug development of MET inhibitors: targeting oncogene addiction and expedience. Nat Rev Drug Discov 2008;7:504–516.
[26] Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, et al. Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 1997;16:68–73.
[27] Michieli P, Basilico C, Pennacchietti S, Maffe A, Tamagnone L, Giordano S, et al. Mutant Met-mediated transformation is ligand-dependent and can be inhibited by HGF antagonists. Oncogene 1999;18:5221–5231.
[28] Nakamura T, Nawa K, Ichihara A. Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem Biophys Res Commun 1984;122:1450–1459.
[29] Nakamura T, Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, et al. Molecular cloning and expression of human hepatocyte growth factor. Nature 1989;342:440–443.
[30] Furlong RA, Takehara T, Taylor WG, Nakamura T, Rubin JS. Comparison of biological and immunochemical properties indicates that scatter factor and hepatocyte growth factor are indistinguishable. J Cell Sci 1991;100:173–177.
[31] Shima N, Nagao M, Ogaki F, Tsuda E, Murakami A, Higashio K. Tumor cytotoxic factor/hepatocyte growth factor from human fibroblasts: cloning of its cDNA, purification and characterization of recombinant protein. Biochem Biophys Res Commun 1991;180:1151–1158.
[32] Kosai K, Matsumoto K, Nagata S, Tsujimoto Y, Nakamura T. Abrogation of Fas-induced fulminant hepatic failure in mice by hepatocyte growth factor. Biochem Biophys Res Commun 1998;244:683–690.
[33] Amicone L, Spagnoli FM, Spath G, Giordano S, Tommasini C, Bernardini S, et al. Transgenic expression in the liver of truncated Met blocks apoptosis and permits immortalization of hepatocytes. EMBO J 1997;16:495–503.
[34] Wang L, Wang X, Xie G, Wang L, Hill CK. DeLeve LD Liver sinusoidal endothelial cell progenitor cells promote liver regeneration in rats. J Clin Invest 2012;122:1567–1573.
[35] Huh CG, Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. Proc Natl Acad Sci U S A 2004;101:4477–4482.
[36] Borowiak M, Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. Proc Natl Acad Sci U S A 2004;101:10608–10613.
[37] Giebeler A, Boekschoten MV, Klein C, Borowiak M, Birchmeier C, Gassler N, et al. C-Met confers protection against chronic liver tissue damage and fibrosis progression after bile duct ligation in mice. Gastroenterology 2009;137:308.
[38] Aberger F, Schmidt G, Richter K. The Xenopus homologue of hepatocyte growth factor-like protein is specifically expressed in the presumptive neural plate during gastrulation. Mech Dev 1996;54:23–37.
[39] Takeo S, Arai H, Kusano N, Harada T, Furuya T, Kawauchi S, et al. Examination of oncogene amplification by genomic DNA microarray in hepatocellular carcinomas: comparison with comparative genomic hybridization analysis. Cancer Genet Cytogenet 2001;130:127–132.
[40] Kondo S, Ojima H, Tsuda H, Hashimoto J, Morizane C, Ikeda M, et al. Clinical impact of c-Met expression and its gene amplification in hepatocellular carcinoma. Int J Clin Oncol 2013;18:207–213.
[41] Wang K, Lim HY, Shi S, Lee J, Deng S, Xie T, et al. Genomic landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular carcinoma. Hepatology 2013;58:706–717.
[42] Guichard C, Amaddeo G, Imbeaud S, Ladeiro Y, Pelletier L, Maad IB, et al. Integrated analysis of somatic mutations and focal copy-number changes identifies key genes and pathways in hepatocellular carcinoma. Nat Genet 2012;44:694–698.
[43] Jia CC, Wang TT, Liu W, Fu BS, Hua X, Wang GY, et al. Cancer-associated fibroblasts from hepatocellular carcinoma promote malignant cell proliferation by HGF secretion. PLoS One 2013;8:e63243.
[44] Ueki T, Fujimoto J, Suzuki T, Yamamoto H, Okamoto E. Expression of hepatocyte growth factor and its receptor c-met proto-oncogene in hepatocellular carcinoma. Hepatology 1997;25:862–866.
[45] Boix L, Rosa JL, Ventura F, Castells A, Bruix J, Rodes J, et al. C-met mRNA overexpression in human hepatocellular carcinoma. Hepatology 1994;19:88–91.
[46] Suzuki K, Hayashi N, Yamada Y, Yoshihara H, Miyamoto Y, Ito Y, et al. Expression of the c-met protooncogene in human hepatocellular carcinoma. Hepatology 1994;20:1231–1236.
[47] Noguchi O, Enomoto N, Ikeda T, Kobayashi F, Marumo F, Sato C. Gene expressions of c-met and hepatocyte growth factor in chronic liver disease and hepatocellular carcinoma. J Hepatol 1996;24:286–292.
[48] Kiss A, Wang NJ, Xie JP, Thorgeirsson SS. Analysis of transforming growth factor (TGF)-alpha/epidermal growth factor receptor, hepatocyte growth Factor/c-met, TGF-beta receptor type II, and p53 expression in human hepatocellular carcinomas. Clin Cancer Res 1997;3:1059–1066.
[49] Tavian D, De PG, Benetti A, Portolani N, Giulini SM, Barlati S. U-PA and c-MET mRNA expression is co-ordinately enhanced while hepatocyte growth factor mRNA is down-regulated in human hepatocellular carcinoma. Int J Cancer 2000;87:644–649.

[50] Daveau M, Scotte M, Francois A, Coulouarn C, Ros G, Tallet Y, et al. Hepatocyte growth factor, transforming growth factor alpha, and their receptors as combined markers of prognosis in hepatocellular carcinoma. Mol Carcinog 2003;36:130–141.
[51] Suzuki M, Shiraha H, Fujikawa T, Takaoka N, Ueda N, Nakanishi Y, et al. Des-gamma-carboxy prothrombin is a potential autologous growth factor for hepatocellular carcinoma. J Biol Chem 2005;280:6409–6415.
[52] Gao J, Feng X, Inagaki Y, Song P, Kokudo N, Hasegawa K, et al. Des-gamma-carboxy prothrombin and c-Met were concurrently and extensively expressed in hepatocellular carcinoma and associated with tumor recurrence. Biosci Trends 2012;6:153–159.
[53] Weitz IC, Liebman HA. Des-gamma-carboxy (abnormal) prothrombin and hepatocellular carcinoma: a critical review. Hepatology 1993;18:990–997.
[54] Kaposi-Novak P, Lee JS, Gomez-Quiroz L, Coulouarn C, Factor VM, Thorgeirsson SS. Met-regulated expression signature defines a subset of human hepatocellular carcinomas with poor prognosis and aggressive phenotype. J Clin Invest 2006;116:1582–1595.
[55] Shiota G, Rhoads DB, Wang TC, Nakamura T, Schmidt EV. Hepatocyte growth factor inhibits growth of hepatocellular carcinoma cells. Proc Natl Acad Sci U S A 1992;89:373–377.
[56] Ogasawara H, Hiramoto J, Takahashi M, Shirahama K, Furusaka A, Hiyane S, et al. Hepatocyte growth factor stimulates DNA synthesis in rat preneoplastic hepatocytes but not in liver carcinoma cells. Gastroenterology 1998;114:775–781.
[57] Nakanishi K, Fujimoto J, Ueki T, Kishimoto K, Hashimoto-Tamaoki T, Furuyama J, et al. Hepatocyte growth factor promotes migration of human hepatocellular carcinoma via phosphatidylinositol 3-kinase. Clin Exp Metastasis 1999;17:507–514.
[58] Shirako E, Hirayama N, Tsukada Y, Tanaka T, Kitamura N. Up-regulation of p21CIP1 expression mediated by ERK-dependent and -independent pathways contributes to hepatocyte growth factor-induced inhibition of HepG2 hepatoma cell proliferation. J Cell Biochem 2008;104:176–188.
[59] Zhang SZ, Pan FY, Xu JF, Yuan J, Guo SY, Dai G, et al. Knockdown of c-Met by adenovirus-delivered small interfering RNA inhibits hepatocellular carcinoma growth in vitro and in vivo. Mol Cancer Ther 2005;4:1577–1584.
[60] Salvi A, Arici B, Portolani N, Giulini SM, De PG, Barlati S. In vitro c-met inhibition by antisense RNA and plasmid-based RNAi down-modulates migration and invasion of hepatocellular carcinoma cells. Int J Oncol 2007;31:451–460.
[61] Xie B, Xing R, Chen P, Gou Y, Li S, Xiao J, et al. Down-regulation of c-Met expression inhibits human HCC cells growth and invasion by RNA interference. J Surg Res 2010;162:231–238.
[62] Schmidt C, Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, et al. Scatter factor/hepatocyte growth factor is essential for liver development. Nature 1995;373:699–702.
[63] Uehara Y, Minowa O, Mori C, Shiota K, Kuno J, Noda T, et al. Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor. Nature 1995;373:702–705.
[64] Takami T, Kaposi-Novak P, Uchida K, Gomez-Quiroz LE, Conner EA, Factor VM, et al. Loss of hepatocyte growth factor/c-Met signaling pathway accelerates early stages of N-nitrosodiethylamine induced hepatocarcinogenesis. Cancer Res 2007;67:9844–9851.
[65] Marx-Stoelting P, Borowiak M, Knorpp T, Birchmeier C, Buchmann A, Schwarz M. Hepatocarcinogenesis in mice with a conditional knockout of the hepatocyte growth factor receptor c-Met. Int J Cancer 2009;124:1767–1772.
[66] Tward AD, Jones KD, Yant S, Kay MA, Wang R, Bishop JM. Genomic progression in mouse models for liver tumors. Cold Spring Harb Symp Quant Biol 2005;70:217–224.
[67] Liu ML, Mars WM, Michalopoulos GK. Hepatocyte growth factor inhibits cell proliferation in vivo of rat hepatocellular carcinomas induced by diethylnitrosamine. Carcinogenesis 1995;16:841–843.
[68] Yaono M, Hasegawa R, Mizoguchi Y, Futakuchi M, Nakamura T, Ito N, et al. Hepatocyte growth factor enhancement of preneoplastic hepatic foci development in rats treated with diethylnitrosamine and N-ethyl-N-hydroxyethylnitrosamine. Jpn J Cancer Res 1995;86:718–723.
[69] Shiota G, Wang TC, Nakamura T, Schmidt EV. Hepatocyte growth factor in transgenic mice: effects on hepatocyte growth, liver regeneration and gene expression. Hepatology 1994;19:962–972.
[70] Horiguchi N, Takayama H, Toyoda M, Otsuka T, Fukusato T, Merlino G, et al. Hepatocyte growth factor promotes hepatocarcinogenesis through c-Met autocrine activation and enhanced angiogenesis in transgenic mice treated with diethylnitrosamine. Oncogene 2002;21:1791–1799.
[71] Santoni-Rugiu E, Preisegger KH, Kiss A, Audolfsson T, Shiota G, Schmidt EV, et al. Inhibition of neoplastic development in the liver by hepatocyte growth factor in a transgenic mouse model. Proc Natl Acad Sci U S A 1996;93:9577–9582.
[72] Shiota G, Kawasaki H, Nakamura T, Schmidt EV. Characterization of double transgenic mice expressing hepatocye growth factor and transforming growth factor alpha. Res Commun Mol Pathol Pharmacol 1995;90:17–24.
[73] Bussolino F, Di Renzo MF, Ziche M, Bocchietto E, Olivero M, Naldini L, et al. Hepatocyte growth factor is a potent angiogenic factor which stimulates endothelial cell motility and growth. J Cell Biol 1992;119:629–641.
[74] Zhang YW, Su Y, Volpert OV, Vande Woude GF. Hepatocyte growth factor/scatter factor mediates angiogenesis through positive VEGF and negative thrombospondin 1 regulation. Proc Natl Acad Sci U S A 2003;100:12718–12723.
[75] Eckerich C, Zapf S, Fillbrandt R, Loges S, Westphal M, Lamszus K. Hypoxia can induce c-Met expression in glioma cells and enhance SF/HGF-induced cell migration. Int J Cancer 2007;121:276–283.
[76] Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 2003;3:347–361.
[77] Rose SD, Aghi MK. Mechanisms of evasion to antiangiogenic therapy in glioblastoma. Clin Neurosurg 2010;57:123–128.
[78] Huynh H, Ong R, Soo KC. Foretinib demonstrates anti-tumor activity and improves overall survival in preclinical models of hepatocellular carcinoma. Angiogenesis 2012;15:59–70.
[79] Martell RE, Puzanov I, Ma WW, Santoro A, Dy GK, Goff LW, et al. Safety and efficacy of MET inhibitor tivantinib (ARQ 197) combined with sorafenib in patients (pts) with hepatocellular carcinoma (HCC) from a phase I study (abstract). J Clin Oncol 2012;30:abstr 4117.
[80] Munshi N, Jeay S, Li Y, Chen CR, France DS, Ashwell MA, et al. ARQ 197, a novel and selective inhibitor of the human c-Met receptor tyrosine kinase with antitumor activity. Mol Cancer Ther 2010;9:1544–1553.
[81] Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. Lancet Oncol 2013;14:55–63.
[82] Katayama R, Aoyama A, Yamori T, Qi J, Oh-Hara T, Song Y, et al. Cytotoxic activity of tivantinib (ARQ 197) is not due solely to c-MET inhibition. Cancer Res 2013;73:3087–3096.
[83] Basilico C, Pennacchietti S, Vigna E, Chiriaco C, Arena S, Bardelli A, et al. Tivantinib (ARQ197) displays cytotoxic activity that is independent of its ability to bind MET. Clin Cancer Res 2013;19:2381–2392.
[84] O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, et al. Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med 2011;364:205–214.
[85] Liu X, Shi Y, Maag DX, Palma JP, Patterson MJ, Ellis PA, et al. Iniparib nonselectively modifies cysteine-containing proteins in tumor cells and is not a bona fide PARP inhibitor. Clin Cancer Res 2012;18:510–523.
[86] Patel AG, De Lorenzo SB, Flatten KS, Poirier GG, Kaufmann SH. Failure of iniparib to inhibit poly(ADP-Ribose) polymerase in vitro. Clin Cancer Res 2012;18:1655–1662.
[87] Yau T, Sukeepaisarnjaroen W, Chao Y, Yen C, Lausoontornsiri W, Chen P, et al. A phase I/II study of foretinib, an oral multikinase inhibitor targeting MET, RON, AXL, TIE-2, and VEGFR in advanced hepatocellular carcinoma (HCC). J Clin Oncol 2012;30:4108.
[88] Verslype C, Cohn AL, Kelley RK, Yang T, Su W, Ramies DA, et al. Activity of cabozantinib (XL184) in hepatocellular carcinoma: Results from a phase II randomized discontinuation trial (RDT). J Clin Oncol 2012;30:4007.
[89] Selden C, Farnaud S, Ding SF, Habib N, Foster C, Hodgson HJ. Expression of hepatocyte growth factor mRNA, and c-met mRNA (hepatocyte growth factor receptor) in human liver tumours. J Hepatol 1994;21:227–234.